Resistance of Melligen cells to pro-inflammatory cytokines involved in beta cell death

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

JANET LAWANDI

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Presentations

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Abstract

It has been shown that stable transfection of insulin cDNA into the human liver cell line Huh7 resulted in synthesis, storage and regulated release of insulin to a glucose stimulus (Huh7ins cells). However, Huh7ins cells responded to glucose at sub-physiological levels (2.5mM) and in order to maintain normoglycaemia, insulin secretion in response to physiological levels of glucose is required. Consequently, the Huh7ins cells were further transfected with the human glucokinase gene to correct the skewed insulin secretory profile. The resulting cell line (Melligen) responds to glucose in the 4-5mM physiological range.

If Melligen cells are to be used clinically to correct patient blood glucose concentrations, they need to be resistant to the toxic effects of pro-inflammatory cytokines responsible for the immune-mediated destruction of beta cells. Pro-inflammatory cytokines such as interferon-gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) play a major role in beta cell elimination during diabetes development. The aim of the present study was to determine if Melligen cells were resistant to the toxic effects of these cytokines.

Cells were exposed to IFN-γ (384ng/mL), TNF-α (10ng/mL) and IL-1β (2ng/mL) for up to 10 days. Cell viability was measured using the Annexin V/propidium iodide (PI), PI and MTT cell viability assays. Insulin storage and chronic secretion were measured by radioimmunoassay. Acute insulin secretion was also determined by static incubations with increasing concentrations of 0, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5 and 20mM glucose. Nitric oxide levels were assayed by the Griess reaction. The glucose-responsive beta cell line, MIN-6, was used as a positive control throughout. Expression of the cytokine receptors IFNR1, IFNR2, IL1R1, IL1R2, TNFR1 and TNFR2 was determined in human pancreatic islet cells and the liver cell lines cultured in the absence of the cytokine treatment by RT-PCR. The quantitative analysis of the inhibitors of NF-κB (IκB-α, IκB-β IκB-ε) and NF-κB downstream effectors, iNOS, MCP-1 and Fas, was determined by real time RT-PCR in cytokine treated Huh7ins and Melligen cells with islet cells used as a positive control. Microarray analysis was used to determine which gene networks were being induced after 1h or 24h cytokine treatment of Melligen cells. Melligen cells were also tested for suitability for implantation. They were encapsulated (AustriaNova, Singapore) and insulin secretion

and glucose responsiveness were determined. The cells were also treated with a single or double dose of 100μM or 20mM STZ to determine susceptibility to this beta cell toxin.

The viability of MIN-6 cells was reduced after 2 days of culture with cytokines ($P< 0.05$). In contrast, the viability of Huh7, Huh7ins and Melligen cells was unaffected by cytokine treatment over 10 days. In addition to this, flow cytometry results corroborated the fact that there was no apoptotic cell death in these cell lines. The triple cytokines did not diminish chronic insulin secretion, storage or the glucose-responsiveness of Melligen cells even after 10 days of co-culture. After exposure to cytokines MIN-6 cells also produced higher levels of nitric oxide as compared to Huh7ins cells ($P<0.05$). Expression of cytokine receptors IFNR1, IFNR2, IL1R1, IL1R2 and TNFR1 was detected in pancreatic islet cells and all liver cell lines. However, there was an absence of cytokine receptor TNFR2 expression in the liver cell lines. Real time RT-PCR results revealed down-regulation of the inhibitors of NF-κB and Fas in Huh7ins and Melligen cells, which is in contrast to the molecular mechanism seen in islet cells. The liver cell lines expressed very low levels of iNOS and there was no expression of MCP-1 detected by RT-PCR. Microarray analysis also revealed a network of genes up-regulated by NF-κB. Encapsulation of Melligen cells did not affect insulin function of the cells and the double dose 100μM STZ treatment did not affect the cells ($P>0.05$).

In summary, Huh7ins and Melligen cells were more resistant to the toxic effects of pro-inflammatory cytokines as compared to pancreatic beta cells most likely by NF-κB dependent pathways. These findings suggest that insulin-secreting hepatocytes will be less susceptible to destruction by the autoimmune process that eliminates beta cells in Type 1 diabetes development.

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